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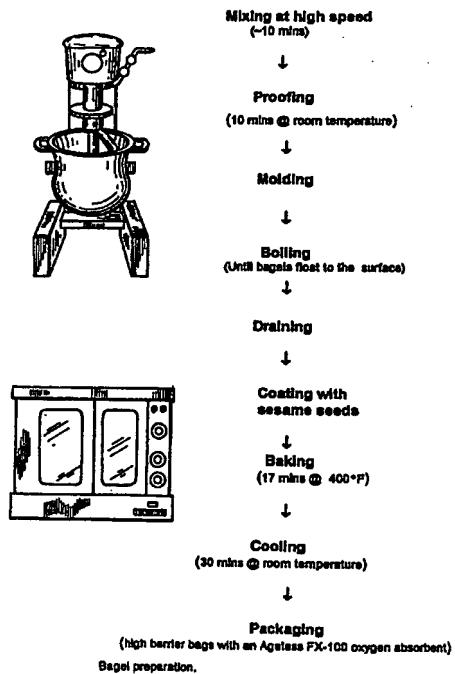
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(54) Title: COMBINED REFORMULATION/PACKAGING TO DELAY STALING IN BAKERY PRODUCTS

**(57) Abstract**

Spoilage, by staling, is a major problem in the bakery industry. This problem is overcome by a method for delaying staling in bakery products, which synergistically combines reformulation and modified atmosphere packaging. Dough is reformulated with an anti-staling ingredient, such as an enzyme, a gum and high fructose corn syrup. The reformulated dough is subsequently baked and packaged in a gaseous environment such as CO<sub>2</sub> or a mixture of gases such as N<sub>2</sub> and CO<sub>2</sub> or in the presence of an oxygen absorbent. With this method, staling may be delayed up to 42 days.



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COMBINED REFORMULATION/PACKAGING TO DELAY STALING  
IN BAKERY PRODUCTS

BACKGROUND OF THE INVENTION

The present invention relates to a method for delaying staling in bakery products, which synergistically combines reformulation and modified atmosphere packaging.

The importance of the bakery industry is well known worldwide. Bakery products are an important source of nutrients in our diet. Consumption of bakery products in North America is estimated at \$23 billion dollars annually with roughly 50% being spent on bread and rolls (Peat Marwick Group, 1991). However, spoilage occurs shortly after baking. The two main sources of such spoilage are essentially microbial spoilage, more specifically mold growth spoilage, and staling.

Microbial spoilage comprises bacterial, yeast and mold spoilage. All microorganisms require three basic elements: food, temperature and moisture. Pre-packaged bakery products provide conditions conducive to microbial growth. Mold spoilage is responsible for the majority of losses in the bakery industry in the United States.

The majority of the molds found in white bread belong to the genus *Aspergillus* and *Penicillium*. Other mold species e.g., *Rhizopus*, *Monilia*, and *Mucor* species have also been implicated. According to Bullerman and Hartung (1973), aflatoxin producing molds have never been detected in either flour or bread. They also stated that flour contained more toxic molds than bread, due to the fact that mold spores are not very heat resistant.

Thus, mold spoilage results from postprocessing contamination. This occurs during cooling and packaging from contamination by airborne spores or contact with contaminated surfaces. Contamination also results from food handlers and raw ingredients such as glazes, nuts, spices and sugars. Under warm humid conditions, mold problems are even more troublesome and mold growth is visible within 48 hours after baking and packaging.

As aforesaid, just after mold spoilage, staling is the second main source of spoilage in the bakery industry.

Several definitions of staling have been proposed. Bechtel et al. (1953) defined staling as a "decreasing consumer acceptance of bakery product caused by changes in the crumb and the crust other than those resulting from the action of spoilage microorganisms". According to Bechtel and Meisner (1951) consumers view staling as hardening of the crumb which has a dry mouth feel, an increase in crumbliness, a loss of flavour and aroma, and a softening and toughening of the crust". Kuip (1979) stated that staling was "the gross changes and the various underlying reactions, as well as other physical or chemical phenomena which contribute to the subjective estimate known as staling".

By definition, bread staling refers to all the changes that occur in bread after baking. The consumer perceives staling of bread by changes in the aroma, toughening of the crust and, most importantly, firming of the crumb. Based on market studies, the wholesale baking industry believes that consumers equate "squeeze" softness with freshness and make their choice

at the supermarket bread rack accordingly. Thus, the bakery industry attempts to produce the most "squeezable" bread. Objective measurements of staling are complicated since "staleness of bread is a subjective quality which is ultimately assessed by the senses" (Toufeili et al., 1994). Under optimal storage conditions, bread "stales" after 2 to 3 days on supermarket shelves.

Staling can be divided into crust staling and crumb staling. The majority of research has focused on crumb staling as crust staling seems impossible to prevent.

Crust staling is due to moisture migration from the crumb to the crust and from absorption of moisture from the atmosphere if the relative humidity (RH) is high, i.e.  $RH > 80\%$ . If the bread is left unpacked, it dries out completely. If packaged, the crust soon stale. Crust staling is enhanced by high moisture barrier packaging materials which do not permit moisture to pass from the crumb to the atmosphere. Thus, it remains in the crust.

Crumb staling is an even more complex phenomenon. The crumb becomes firmer, less elastic, crumblier, harsh textured, and it has a dry mouth feel.

As can be appreciated, methods to control staling are therefore of a great importance to the bakery industry. As a matter of fact, staling results in millions of dollars of lost revenue annually. It was already reported that returns due to staling in the United States are 8%, accounting for almost 50 million kg of product returns annually. To overcome this major spoilage

problem, staling of bakery products has been the subject of extensive investigation.

Two approaches to delay staling has been investigated so far. The first approach is through packaging under a modified atmosphere involving elevated CO<sub>2</sub> levels. While packaging under 100% CO<sub>2</sub> delays both mold growth and staling for 6 weeks, products are rejected by consumers after 4 weeks due to the sharp acidic taste of CO<sub>2</sub> dissolved in the aqueous phase of the product.

The other and more successful approach is through reformulation involving Guar gum, high fructose corn syrup (HFCS) and enzymes. It has been shown that a highly acceptable product from both a textural and sensorial viewpoint could be produced commercially through appropriate levels of enzyme, gum and HFCS in the formulation.

#### SUMMARY OF THE INVENTION

In accordance with the invention, it has been found that combined reformulation and packaging offers the baking industry a viable approach to inhibit the two main potential spoilage concerns viz. staling and mold growth.

Thus, the present invention provides a method for delaying staling in a bakery product containing a dough made of a flour for up to 42 days, which comprises the steps of:

-reformulating the dough by adding to the flour an ingredient for delaying firmness of the baked product;

- baking the reformulated dough to form the requested bakery product; and
- packaging the bakery product in a modified atmosphere to prevent microbial spoilage.

Thus the present invention combines both of these known technologies. Such a combination has proved to be synergistic. As a matter of fact, it has been found that staling and mold growth can be prevented/delayed in bagels and any other bakery products for up to 6 weeks or more through appropriate reformulation and modified atmosphere packaging (MAP).

The estimated cost for combining both of these technologies in order to obtain the requested shelf life extension is 20 to 30 cents/dozen bagels. However, the increased cost should easily be defrayed through less returns and downgrading of products to croutons, less production costs through bulk processing/packaging and most importantly extended shelf life, market growth and increased profitability.

The invention and its advantages will be better understood upon reading the following non-restrictive description made upon reference to the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1. is a schematic representation of a standard bagel preparation.

FIG. 2. is a graphical representation of the response surface of enzyme and guar during a texture evaluation.

FIG. 3. is a graphical representation of the response surface of enzyme and guar gum during a sensory evaluation.

FIG. 4 is a graphical representation of the compressibility of control bagels at different days.

FIG. 5 is a graphical representation of the compressibility of bagels treated with Novamyl enzyme.

FIG.6 is a graphical representation of the compressibility of bagels treated with Superfresh enzyme.

FIG.7 is a graphical representation of the compressibility of bagels treated with Megafresh enzyme.

FIG.8 is a graphical representation of the compressibility of bagels treated with guar gum.

FIG. 9 is a graphical representation of the compressibility of bagels treated with high fructose corn syrup.

#### DETAILED DESCRIPTION OF THE INVENTION

##### REFORMULATION

Reformulation by means of one or more anti-staling ingredients is a well known technology that has been developed to retard staling. The reformulation ingredients that are commonly used, include shortenings, mono and diglycerides, surfactants, enzymes, gums, gluten free flour (see Table 1). However, only enzymes, gums and high fructose corn syrups (HFCS) will be discussed hereinafter in detail.

**TABLE 1:** Ingredients commonly used in the reformulation and their percentages.

Ingredients	Trade Name	Supplier	Percentages used <sup>1</sup>
<b><u>Enzymes:</u></b>			
Genetically modified maltogenic $\alpha$ -amylase	Novamyl	Novo Nordisk (Danbury, CT)	0.031, 0.047
Fungal and bacterial $\alpha$ -amylases	Superfresh plus	Enzyme Biosystems (Beloit, WI)	0.1, 0.15, 0.2
Bacterial $\alpha$ -amylase and glucotransferase	Megafresh plus	Enzyme Biosystems (Beloit, WI)	0.1, 0.15, 0.2
<b><u>Gums:</u></b>			
Guar	Guar SG25	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Xanthan	Xanthan 100	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Locust bean	LBG SG14	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Agar	Agar Agar	Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
Cellulose	Cellulose 40	Soca Floc (Chicago, IL)	1
	Cellulose 300	Soca Floc (Chicago, IL)	1
	Cellulose 900	Soca Floc (Chicago, IL)	1
Methylcellulose	Methocel	Dow ingredients (Midland, MI)	1
Algin	Kelvis	Kelco (Chicago, IL)	0.2, 0.6, 1
Pectin	Classic AB201	Herbstreith & Fox/ Amcan Ingredients (Lachine, QC)	0.2, 0.6, 1
<b><u>Syrups<sup>2</sup>:</u></b>			
HFCS Liquid	HFCS Liquid	ADM Corn Processing (Decatur, AL)	50, 100
HFCS Granular	HFCS Granular	ADM Corn Processing (Decatur, AL)	50, 100
<b><u>Flour:</u></b>			
Rice	Instant Rice Flour	IGT (Lincoln, NE)	25
Barley	Instant Barley Flour	IGT (Lincoln, NE)	25
Corn	Instant Corn Flour	IGT (Lincoln, NE)	50
<b><u>Surfactants:</u></b>			
Sodium Stearyl Lactylate	Atlas SSL	ICI Surfactants (Lachine, QC)	0.25, 0.375
SSL and amylase	Atlas p51	ICI Surfactants (Lachine, QC)	0.25, 0.375

1: Based on a flour weight basis.

2: Based on a sugar replacement basis.

a) Enzymes

Many enzymes can be added to dough to enhance its properties. The major enzymes commercially used in bread dough are  $\alpha$ -amylases,  $\beta$ -amylases, invertases, maltases, zymases and proteinases (see Table 2).

Table 2 :: Major enzymes used in dough fermentation.

ENZYME	SOURCE	ACTS ON	PRODUCTS
$\alpha$ -amylase	Flour, Fungal preparation Malt Bacterial preparation	Starch	Soluble starch and dextrins
$\beta$ -amylase	Flour Malt	Dextrins	Maltose
Invertase	Yeast	Sucrose	Invert sugar
Maltase	Yeast	Maltose	Dextrose
Zymase	Yeast	Invert sugar Dextrose	Carbon dioxide Alcohol Flavors
Proteinase	Flour Fungal preparation Bacterial preparation	Gluten	Enables faster mixing and improved dough extensibility

Other enzymes which could be used include lipoxygenases, pentosanases and others.

Since amylases and glucoamylases are most often used, their mode of action will be discussed in more detail.

Amylases are divided into  $\alpha$ -amylases and  $\beta$ -amylases. They can be of different sources: bacterial, fungal or cereal. Different sources give enzymes different properties. Amylases are usually added to increase the level of fermentable sugars, to increase the production of simple sugars leading to a sweeter product with a better colour, since the reducing sugars produced react with other components in bread to give Maillard reaction products. They also improve gas and moisture retention

properties of the dough. Furthermore, heat stable amylases retard bread staling.

It has already been reported that small amounts of bacterial amylases had a beneficial softening effect in bread whereas high levels resulted in unacceptable softness. The main advantage of bacterial  $\alpha$ -amylase is its thermostability since its action occurs once starch has gelatinized. It has also been reported that fungal, cereal and bacterial amylases result in softer bread. Bacterial amylases do not affect the initial bread firmness, but reduce the firming rate during storage. Conversely, fungal amylases, decrease the initial bread firming but do not affect the firming rate.

$\alpha$ -Amylases are the most widely used enzymes. Bacterial  $\alpha$ -amylases survive baking in contrast to cereal and fungal enzymes and are commercially used as antistaling agents. However, excessive amounts can produce adverse effects during storage. Bread can turn gummy and lose desirable textural properties due to the thermostable property of the enzyme. New improved bacterial amylases with reduced thermostability have been introduced to prevent these problems occurring during storage.

Bacterial  $\alpha$ -amylase cleave linkages in the amorphous regions of starch where they are most accessible to enzyme attack. Once the enzyme complexes with the starch molecule and the initial cleavage has been made, the enzyme may remain with one fragment and produce one or more breaks before dissociating and moving to another substrate molecule. Prior to baking, they only digest the damaged starch (5%). On the other hand, bacterial

and fungal  $\alpha$ -amylases produce small dextrins that interfere with hydrogen bonds formation in starch protein interaction and, thus, retard bread firming.

$\beta$ -Amylase is an exoenzyme. It releases two joined glucose unit (maltose) from starch.  $\beta$ -Amylase is normally present in flour so that addition supplementation is not required. Still, the addition of amylase will enhance the action of  $\beta$ -amylases since it will produce small dextrins on which  $\beta$ -amylase can readily act.

Glucoamylase is an exoenzyme which works on the nonreducing end of a starch chain and releases glucose molecules in a step wise process. It used in bread for glucose production since it results in a sweeter product compared to maltose produced by  $\beta$ -amylase.

Two other major groups of enzymes can also be used: non-starch polysaccharide degrading enzymes, and lipid modifying enzymes. The non-starch polysaccharide enzymes consist mainly of hemicellulases and pentosanases that have been shown to have some effect retarding staling. The lipid modifying enzymes group include lipoxygenases lipases and phospholipases. These have also been the subject of many studies and appear to have an effect on bread firming. The action of lipoxygenase such as soy lipoxygenase, appear to be related with gluten development. It was proposed that the action of lipoxygenase involves modification of the hydrophobic areas of the gluten. It was assumed that the release of gluten bound lipids will provide additional free lipids for complexing with starch during baking leading to a softer bread.

The reduction in bread firmness due to enzymatic action has already been reported in the literature. It has been shown that bread supplemented with bacterial amylases is the softest during storage. It has also been reported that bread supplemented with barley, malt or fungal enzyme showed the same initial softness as the fresh product. Furthermore, it has been observed an order of decreasing degree of starch crystallinity from bacterial  $\alpha$ -amylase, cereal  $\alpha$ -amylase, fungal  $\alpha$ -amylase and unsupplemented bread, postulating that the degree of crystallinity paralleled the heat stability of the enzyme, which produce lower molecular weight starch units. These will have more freedom of movement and can more easily arrange themselves into lattice position. Thus, starch crystallinity and bread firming are not synonymous.

It has also been reported that bacterial  $\alpha$ -amylase and  $\beta$ -amylase inhibit bread from firming during five days of storage. Bread supplemented with amylases contains great quantities of dextrans which appear to have an anti-firming effect. Bacterial amylases reduces the firming rate of bread and the rate of firming increases with increasing concentration of enzyme confirming our observations. However, an excessive amount of the enzymes could lead to keyholing (weakness of loaf side walls). However, this defect was not observed for bagels.

Most studies to date have examined the effect of softening agents individually. However, combination treatments with these agents could have a more pronounced effect on staling.

b) Gums

The effect of gums on staling have not been investigated extensively. Moreover, it seems that they also play an important role as anti-staling agents.

A variety of gums can be used to increase the keeping quality of bakery products. When incorporated into a baked good formulation, gums have the ability to bind water into a gel to reduce water migration and to control rheological properties resulting in an extended shelf life. This extension of freshness can be attributed to the ability of gums to immobilize and bind water as well as interfere with hydrogen bonding between starch and protein i.e., the "bound" water exerts a plasticizing effect.

Examples of gums include guar, xanthan, locust bean gum, agar gum, cellulose, methylcellulose, alginates and pectins. Of course such gums may vary in their chemical structure and in their ability to bind water and to maintain freshness in a product.

Guar gum is a polysaccharide with a straight chain of D-mannopyranose units joined by linkages with a side branching unit of a single D-galactopyranose unit joined to every other mannose unit by a (1,6) linkages. It has a high hydration and water binding capacities, and forms a viscous colloidal solutions when hydrated in cold water systems.

Xanthan gum is a high molecular weight polysaccharide produced by the action of micro-organism on dextrose. It is very heat stable, it has a high moisture binding capacity and it

contributes to the elasticity of the dough and shelf life extension of baked products.

c) HFCS

High fructose corn syrup (HFCS) can provide shelf life extension by enhancing the water retention of baked goods. HFCS are humectants which retain moisture in the crumb, thereby resulting in a less firm, less stale fresher product.

HFCS is a bright, transparent liquid. It is produced by treating high conversion corn syrup with immobilized glucose isomerase, an enzyme that catalyses the rearrangement of the sugar molecule from the aldose to the ketose form. The transformation involves an intermolecular transfer of hydrogen between adjacent carbon atoms to convert glucose to fructose. The high level of fructose gives its hygroscopic and sweet properties. Thus, it could affect staling by binding the moisture and/or by interfering with the hydrogen bond formation between protein and starch. However, at higher levels of use, it can cause stickiness and may adhere to packaging materials upon storage.

MODIFIED ATMOSPHERE PACKAGING (MAP)

Studies to date have focused on formulation changes to delay staling and enhance product shelf life. However, other factors such as storage atmosphere, storage temperature and method of production (i.e., retarding or nonretarding) may also influence the texture of the product. Actually, several studies have shown that gas packaging in a CO<sub>2</sub> enriched atmosphere can be used to extend the mold free shelf life of baked products.

Furthermore, other studies have shown that in addition to its antimycotic effect, CO<sub>2</sub> may also have an antistaling effect, although results to date have been contradictory.

The method according to the invention makes use of a modified atmosphere packaging (MAP). MAP has already been defined as "the enclosure of food products in high gas barrier film in which the gaseous environment has been changed or modified to slow respiration rates, reduce microbial growth and retard enzymatic spoilage with the intent of extending shelf life". It is estimated that the demand for MAP foods in North America could reach 11 billion packages the year 2000 (Smith and Simpson, 1995).

MAP is a new packaging technique that makes use of various methods to modify the gas atmosphere surrounding a product, including gas packaging, the use of oxygen absorbents or ethanol vapour generation. As is known, air is composed of about 78% nitrogen (N<sub>2</sub>), 21% oxygen (O<sub>2</sub>), and 1% carbon dioxide (CO<sub>2</sub>). The principle of MAP is that by changing the composition of the atmosphere around a food product, i.e. reducing the amount of O<sub>2</sub> and increasing the levels of CO<sub>2</sub>, one may significantly increase the shelf life of this product.

So far, MAP has been mostly used to increase the shelf life of many food products including bakery products where they were found to extend the mold free shelf life of products. However, it has now been found that MAP also has some effect in delaying staling.

As aforesaid, several methods can be used to modify the gas atmosphere surrounding bakery products. These include vacuum packaging (VP), gas packaging, use of oxygen absorbents and ethanol vapour generators.

Vacuum packaging was the earliest form of MAP. VP is not used for most bakery products since this process causes irreversible deformation of soft products. However, it is used to prevent rancidity problems in short bread.

Gas packaging consists of replacing the air with a gas or a mixture of gases within the package, which is usually an impermeable film. Gases commonly used in MAP are carbon dioxide, nitrogen and carbon monoxide. Other gases, such as chlorine, ethylene oxide, nitrogen oxide, ozone, propylene oxide and sulfur dioxide have been investigated but are not used commercially. The most commonly used gases are N<sub>2</sub> and CO<sub>2</sub> alone or in combination with each other. The reason for this is that they are neither toxic nor dangerous and they are not considered as food additives.

N<sub>2</sub> does not have a antimicrobial effect by itself since it is an inert gas. However, it is usually used as a filler gas to prevent the package collapsing in products that could absorb some CO<sub>2</sub> upon storage. It is also used to prevent rancidity problems in food of low water activity or moisture content i.e., where microbial spoilage is not a problem.

CO<sub>2</sub> is the most important gas since it is both bacteriostatic, fungistatic and can prevent growth of insects in the package. However, it is highly soluble in water and fats, and forms

carbonic acid, resulting in flavour changes when used in high concentrations. Thus, some bakery products can also absorb CO<sub>2</sub> causing the package to collapse.

With MAP alone, spoilage problems due to staling and discolouration still occur. Also, when a product held in a MAP pack is eaten "directly" a bitter flavour of carbonic acid can be noted. This usually appears in the product after four days of storage. Even the N<sub>2</sub> gas produces a noticeable off-odor in bread within one day after baking, an odour which increases with time. Actually, most of the air atmosphere control produce a "stale" odour after seven days at room temperature. However, these odours can be overcome by toasting products prior to consumption.

To overcome this drawback, it has been proposed to use oxygen absorbents. Such absorbents are packaged in gas permeable materials in the form of small pouches, which react chemically with oxygen. Placed in sealed packed containers, they reduce the oxygen concentration to 100 parts per million or even lower and maintain this level, as long as the appropriate packaging film is used. Substances commonly used are iron powder and ascorbic acid. The first oxygen absorbent was an iron powder based absorber developed by Mitsubishi Gas Chemical Company, under the trade name of Ageless in 1977. In 1989, almost 7000 million sachets were sold in Japan with sales of absorbents growing at a rate of 20% per year.

Using oxygen absorbent technology, the mold-free shelf life of white pan bread may be increased 5 days to 45 days at room

temperature while pizza crust has a mold-free shelf life of 14 days at 30°C.

The main problems with oxygen absorbents are consumer resistance to their use in food. Two main consumer concerns are the fear of ingesting the absorbent and the spillage of sachet contents into the food thus adulterating the product. However, oxygen absorbents are inexpensive, non-toxic, fast and easy to use. Hence, the use of an oxygen absorbent is a preservative free method for increasing shelf life and distribution by preventing mold growth.

As aforesaid, most of the studies to date with MAP have focused on extension of the mold free shelf life of products. Studies on the anti-staling effect of enriched CO<sub>2</sub> atmospheres produced conflicting results. Thus, it was observed that the crumb of bread becomes firmer irrespective of the storage atmosphere i.e., storage in air, 100% CO<sub>2</sub> or 100% N<sub>2</sub>. It was also reported that the staling rate of white and whole wheat bread is not significantly reduced when packaged in carbon dioxide or nitrogen as compared to air. It was further reported no clear pattern of firming over time between packaging treatments for pita bread packaged under various atmospheres.

However, it was shown that the compressibility of bread packed under CO<sub>2</sub> is lower than bread packed in air suggesting that carbon dioxide delays bread firming. It was also shown that carbon dioxide significantly decreases compressibility of some baked goods compared to air-stored samples and that softer products were obtained when stored under 100% CO<sub>2</sub>. While the initial compressibility of air and CO<sub>2</sub> stored bread is

identical, bread stored in CO<sub>2</sub> for 72 hours is significantly softer than the air-stored products. Observed differences between water activity of the CO<sub>2</sub> stored samples and air-stored samples after 96 hours of storage suggests that CO<sub>2</sub> atmospheres may affect the water binding in bread.

It has further been reported that CO<sub>2</sub> delays bread staling. Changes in the sorption properties of MAP baked goods are supposedly responsible for this effect. Since amylose is in the crystalline state after one day, amylopectin is the main component with available water binding sites. CO<sub>2</sub> appears to block some of these sites, thereby causing a reduction in hydrogen bonding between the amylopectin branches resulting in a reduced water sorption capacity. Since hydrogen bonding has been shown to result in bread staling, blockage of water binding regions may explain bread firming. The effect of CO<sub>2</sub> was found to exist when water was in "the solute state". The solubility of CO<sub>2</sub> in water is 35 times higher than O<sub>2</sub>. Thus, it is possible that when water is in the solute stage, CO<sub>2</sub> dissolved easily and bound strongly to amylopectin thus preventing hydrogen bonding.

#### COMBINED REFORMULATION AND MODIFIED ATMOSPHERE PACKAGING

As aforesaid, the present invention combines both of the above-mentioned technologies that have been used separately so far, viz. reformulation and MAP.

To determine the effect of the combined reformulation with enzymes, guar, algin and pectin gums and HFCS and modified atmosphere packaging on textural/sensory quality of bagels, a

3 factor, 5 level central composite rotatable design (CCRD) disclosed by Box et al. (1978) was used for fitting second order response surfaces. CCRDs have  $2k+2k+1$  treatment combinations where  $k$  equals the number of variables under study. The experimental design is said to be rotatable since the variance of the predicted response  $Y$ , at designated points ( $X$ 's), is a function only of this distance from the center, rather than a function of the direction. This implies that the variance contours of  $Y$  are concentric circles and a design with this property will leave the variance of  $Y$  unchanged with the design rotated about the center (0,0,0,0) leading to the term rotatable.

In the initial CCRD, (hereinafter called CCRD1), an enzyme (Novamyl), guar gum and HFCS were investigated simultaneously to determine their effect on textural/ sensory quality of bagels. The range of levels of each factor used in the CCRD1 were enzyme (0.0150.075%), guar gum (0.40.8%) and HFCS (15-75%). Variable levels were coded - 2, -1, 0, +1, +2 to facilitate statistical analysis. Values of each level used were based on previous formulation studies. The coded and actual values of enzyme, guar gum and HFCS are shown in Table 3.

**Table 3 : Central Composite Rotatable Design 1: Levels of Novamyl, Guar Gum and High Fructose Corn Syrup.**

Ingredients	Levels (%) <sup>1</sup>				
	-2	-1	0	1	2
Novamyl ( $X_1$ ) 0.015	0.030	0.045	0.060	0.075	
Guar ( $X_2$ ) 0.4	0.5	0.6	0.7	0.8	
HFCS ( $X_3$ ) 15	30	45	60	75	

1- percentage based on flour weight basis except for high fructose corn syrup which was basis on a sugar replacement basis.

For the tests, all bagels were reformulated, baked and packaged in an Ageless FX 100 oxygen absorbent in high gas barrier Cryovac bags (2 bagels /bag). Bagels were stored at ambient temperature and examined for textural/sensory changes over a 42 day storage period as described previously. Bagels were rejected when a compressibility of 0.01 MPa and a sensory score of 3 was reached for each formulation

To test the accuracy of the second order polynomial fitted model to extend both the textural and sensory shelf life of bagels, bagels were reformulated with the desired level of enzyme, gum and HFCS observed at optimum response for each design. Reformulated bagels were again packaged with an Ageless FX100 oxygen absorbent in high gas barrier Cryovac bags (2 bagels/bag) stored at 25°C and monitored for textural sensory changes over a 42 days storage period.

The combined effect of enzyme (Novamyl), guar gum and HFCS on the textural and sensory (overall acceptability) quality of reformulated bagels is shown in Table 4.

Table 4: Central Composite Rotatable Design (CCRD1)

Trials	Levels (%)			Response	
	Novamyl	Guar	HFCS	Texture <sup>1</sup>	Sensory <sup>2</sup>
1	0.030	0.5	30	0.0030	3.6
2	0.060	0.5	30	0.0047	4.0
3	0.030	0.7	30	0.0033	4.2
4	0.060	0.7	30	0.0030	2.8
5	0.030	0.5	60	0.0044	4.0
6	0.060	0.5	60	0.0066	3.0
7	0.030	0.7	60	0.0040	3.6
8	0.060	0.7	60	0.0037	3.6
9	0.015	0.6	45	0.0045	2.6
10	0.075	0.6	45	0.0052	2.8
11	0.045	0.4	45	0.0020	3.2
12	0.045	0.8	45	0.0062	3.2
13	0.045	0.6	15	0.0030	3.2
14	0.045	0.6	75	0.0022	3.0
15	0.045	0.6	45	0.0022	4.2

All tests done in duplicates

1: Compressibility

2: Overall acceptability

The texture evaluation scores ranged from a low of 0.002 MPa (runs # 11,14,15) to 0.0066 MPa (run # 6). The sensory evaluation scores (with the exception of runs 4, 9 and 10) were >3, i.e. the overall acceptability of products was acceptable after 42 days of storage at 25°C. There was a good correlation of 55% between texture and overall acceptability indicating that objective textural measurements were good indicators of product quality and consumer acceptance of the reformulated product.

To quantify the effect of enzyme (Novamyl), guar gum and HFCS on the textural sensory quality of bagels, a RSM approach was used. The second order models resulting from the multiple regression of the uncoded results for texture are:

$$Y_{\text{texture}} = -0.0078 + 0.13X_1 + 0.008X_2 + 0.0002X_3 + 1.06X_1^2 + 0.002X_2^2 - 0.000005X_3^2 - 0.33X_1X_2 + 0.000001X_1X_3 - 0.0001X_2X_3$$

Where  $X_1$ ,  $X_2$  and  $X_3$  stand for an enzyme, a gum and a higher fructose corn syrup respectively, analysis of variance for the fitted model showed that the F-value and the overall correlation coefficient were significant ( $P<0.05$ ) and that the model accounted for 45% of the total variation after being corrected for the mean.

Analysis of least square estimates of the second order polynomial model parameters are shown in Table 5.

Table 5: Analysis of least square estimates of second order polynomial model (parameters for texture).

Model	Estimate	T - Ratio
Intercept ( $b_0$ )	-0.008 (0.008) <sup>a</sup>	-0.96**
Enzyme ( $X_1$ )	0.126 (0.09)	1.32***
Guar Gum ( $X_2$ )	0.0079 (0.02)	0.42***
HFCS ( $X_3$ )	0.002 (0.0001)	2.2***
Enzyme <sup>2</sup> ( $X_1^2$ )	1.05 (0.64)	1.65*
Guar Gum <sup>2</sup> ( $X_2^2$ )	0.01 (0.01)	1.0*
HFCS <sup>2</sup> ( $X_3^2$ )	-0.0001 (0.0001)	-0.88*
Enzyme*Guar Gum ( $X_1X_2$ )	-0.033 (0.11)	-2.95***
Enzyme*HFCS ( $X_1X_3$ )	0.0001 (0.0001)	0.0001 <sub>ns</sub>
Guar Gum*HFCS ( $X_2X_3$ )	-0.0001 (0.0001)	-1.74*
$R^2$ <sup>b</sup> (%)	45	

a: The number in parenthesis is the standard error

b: Coefficient of determination

Level of significance \* P&lt;0.05, \*\* P&lt;0.005, \*\*\* P&lt;0.0005, ns= non significant

Regression analysis of the model showed that the fitted model was highly significant ( $P<0.0005$ ), yet only accounted for 45% of the total variation after being corrected for the means. Examination of the fitted model show that all the linear, quadratic and cross product terms (except enzyme, HFCS,  $X_1X_3$ ) had a significant effect. The significant effect of enzyme or HFCS alone was in agreement with earlier reformulation results. Thus, the combination of these ingredients had a significant effect on staling. This synergistic effect may be explained due to the high carbohydrate content in these treatment combinations and hence a higher water binding and plasticizing effect on crumb texture.

The second order model of the uncoded results for sensory for CCRD1 is:

$$Y_{\text{sensory}} = -11.58 + 196.66X_1 + 33.75X_2 + 0.08X_3 - 1814.81X_1^2 - 28.33X_2^2 - 0.001X_3^2 - 66.66X_1X_2 - 0.000001X_1X_3 + 0.06X_2X_3$$

Where  $X_1$ ,  $X_2$  and  $X_3$  stand for an enzyme, a gum and a high fructose corn syrup respectively, analysis of variance for the fitted model showed that the  $F$  value and the overall correlation coefficient were significant ( $P<0.05$ ) and that the model accounted for 24% of the total variation after being corrected for the mean.

Analysis of least square estimates of the second order polynomial model parameters are shown in Table 6.

Table 6: Analysis of least square estimates of second order polynomial model (parameters for sensory).

Model	Estimate	T - Ratio
Intercept ( $b_0$ )	-11.58 (-2.08) <sup>a</sup>	0.04*
Enzyme ( $X_1$ )	196.66 (3.02)	0.003**
Guar Gum ( $X_2$ )	33.75 (2.64)	0.01*
HFCS ( $X_3$ )	0.08 (1.23)	0.22 <sub>ns</sub>
Enzyme <sup>2</sup> ( $X_1^2$ )	-1814.81 (-4.16)	0.0001***
Guar Gum <sup>2</sup> ( $X_2^2$ )	-28.33 (-2.88)	0.005**
HFCS <sup>2</sup> ( $X_3^2$ )	-0.001 (-3.14)	0.002**
Enzyme*Guar Gum ( $X_1X_2$ )	-66.66 (-0.86)	0.4 <sub>ns</sub>
Enzyme*HFCS ( $X_1X_3$ )	-0.0001 (-0.0001)	1 <sub>ns</sub>
Guar Gum*HFCS ( $X_2X_3$ )	0.06 (0.86)	0.39 <sub>ns</sub>
$R_s^b$ (%)	24	

a: The number in parenthesis is the standard error

b: Coefficient of determination

Level of significance \* P<0.05, \*\* P<0.005, \*\*\* P<0.0005, ns= non significant

It is evident from this table that most of the terms are significant. With the exception of the linear term HFCS ( $X_3$ ), and the cross product terms, all linear and quadratic terms were significant and had a pronounced effect on the overall acceptability of the product. Thus, while the cross products did not influence the overall acceptability of the product, (i.e. taste, odour and texture) they still had an effect on texture of bagels. However, the values of the stationary points for texture and sensory are very close confirming the very high correlation ( $r=0.98$ ) found between the two for texture and compressibility.

The significant linear, quadratic and cross product terms influencing both texture and overall acceptability were subsequently used to generate 3 dimension response surface graphs. These graphs/plots illustrate the important relationship between product variables and their effect on texture and overall acceptability. An example of response surface graphs of enzyme, guar gum and HFCS held constant at a 45% HFCS (sugar replacement basis) for texture and sensory evaluation are shown in Figures 2 and 3 respectively. The response surface graph for

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texture (Figure 2) is an example of a saddle point (Box et al, 1978) where the optimum response is either along the sides or in one or more of the four corners. As Figure 2 illustrates the optimum result i.e., lowest compressibility and hence best texture and less stale product can be achieved by decreasing both the levels of the enzyme and guar gum in the formulation.

However, if the concentration of enzyme or guar gum increases, products will become harder, more stale as shown by increase in compressibility (Figure 1).

Canonical analysis of this set of experimental data indicated that stationary point i.e., point of optimum texture on the fitted surface is neither a minimum or maximum. Actual values of the variables at the stationary point ( $X_0$ ) are shown in Table 7.

Table 7: Actual values of variables at stationary point  $X_0$  (Point of optimum texture).

Variable	Actual Value (%)
Enzyme ( $X_1$ )	0.064
Guar Gum ( $X_2$ )	0.786
HFCS ( $X_3$ )	47.33

All the actual values are well within the experimental range. Furthermore, the predicted value at the stationary point is a compressibility of 0.0043 MPa. When these values are put in the sensory equation the result is a sensory score of 3, i.e. an acceptable score.

Similar trends between these two variables with HFCS held constant at 45% on sensory quality (overall acceptability) of product are shown in Figure 2. Low sensory scores are possible through formulation with low levels of enzyme guar gum or increasing levels of enzyme and guar gum. However, optimum i.e. maximum sensory scores can be achieved by reformulating the product with levels of variables at stationary point shown in Table 8.

Table 8: Actual values of variables at stationary point  $X_0$  (Point of optimum sensory quality).

Variable	Actual Value (%)
Enzyme ( $X_1$ )	0.045
Guar Gum ( $X_2$ )	0.597
HFCS ( $X_3$ )	43.69

Again, all values are within the experimental range. The predicted maximum sensory value (overall acceptability) is 4.47 out of a possible 5 (highly acceptable). These values when plugged into the compressibility equation will result in a compressibility outcome of 0.0061 MPa, which is also highly acceptable.

Response graphs and canonical analysis for flavour and texture all had similar maximum stationary points. Values of variables at the stationary points for both flavour and texture are shown in Table 9.

Table 9: Actual values of variables at stationary point  $X_0$  (Point of optimum sensorial qualities).

Variable	Actual Value (%)	
	Flavor	Texture
Enzyme ( $X_1$ )	0.043	0.045
Guar Gum ( $X_2$ )	0.616	0.597
HFCS ( $X_3$ )	46.04	39.13

These variables levels resulted in flavour and texture scores of 4.2 and 4.4 respectively. Furthermore, there was a significant correlation between flavour, texture and overall acceptability test scores and values at the stationary points, indicating that either one of the tests could be used as an indicator of sensory quality of bagels.

Based on these studies, Novamyl, Guar gum and HFCS at all levels used produced bagels with a 6 weeks textural shelf life and which were also organoleptically acceptable on the basis of odor, flavor and texture at the end of this storage period.

To test the validity of the predicted model (CCRD1) to give a 6 week shelf life, bagels were reformulated with levels of Novamyl, Guar gum

and HFCS shown at the stationary point for both texture and sensory quality of bagels (tables 7 and 8). Bagels were packaged with an oxygen absorbent and monitored for texture and sensory qualities over a 6 weeks period. At the end of this time the average scores for texture and sensory were 0.05 Mpa and 4.2, i.e. reformulated bagels were highly acceptable from both a textural and sensory viewpoint. Furthermore, there was an excellent correlation (98%) between the predicted and actual values for both texture and sensory (overall acceptability) indicating the validity of the RSM approach to predict shelf life.

Surprisingly, such a combination has proved to be synergistic, as will be seen upon reading of the following examples. As can be understood, many changes could be made in the combined reformulation and packaging technique described hereinabove without departing from the scope of the invention.

The invention will be better understood upon reading the following nonrestrictive examples.

During the test reported in these examples, sensory analysis for odor, flavour, texture and overall acceptability was done throughout the 6 weeks storage period using 5 to 10 untrained panelists. Bagels were ranked on days 3, 7, 14, 28 and 42 using a hedonic scale of 1 to 5 where 1=dislike extremely and 5=like extremely. A product was considered unacceptable for each parameter when an average score of 3 was reached. The samples were numbered randomly with 3 numbers to prevent panelists from being biased. The test were conducted in a sensory evaluation room with separated desks, proper lighting and noise reduced to a minimum.

COMPARATIVE EXAMPLE 1 (Reformulation)

For the purpose of comparison, the textural and sensorial changes in non-reformulated (control) bagels were determined and the altered results are reported in Figure 4 and Table 10.

Table 10 : Sensory results for control bagels.

Control	Sensory Analysis												Overall	
	Odor				Flavor				Texture					
	Days of Storage													
Control	7	14	28	42	7	14	28	42	7	14	28	42	7	
	2.2	2.2	1.8	1.6	2.8	2.6	2.4	2.2	2.4	2.2	2.2	2	2.2	
+/	0.4	0.8	0.4	0.5	0.4	0.5	0.5	0.4	0.5	0.4	0.4	0.7	1	
	0.4	0.8	0.4	0.5	0.4	0.5	0.5	0.4	0.5	0.4	0.4	0.7	1	

Average of 5 replicates followed (below) by its Standard Deviation.

All bagels had an initial compression test measurement of 0.008MPa at day 0. This value increased steadily throughout storage from 0.015 to 0.016 MPa as a result of crumb hardening i.e. staling. Based on these results, bagels were deemed stale when a compression test of 0.01 MPa was reached and this was used as the "staling standard" for all reformulated products. However, staling does not just involve moisture migration and crumb hardening but also a loss of flavour components. It is evident that all control bagels had an unacceptable odour, flavour, texture and overall desirability scores (<3) after 3 days only. Therefore, while a 6 weeks mold free shelf life is possible using oxygen absorbent technology staling is still a major problem limiting the shelf life of bagels. This problem can be addressed through reformulation with enzymes, gums and high fructose corn syrups.

Bagels were reformulated by adding ingredients to a Hobart mixer (D300, Hobart Canada Inc., Don Mills, Ontario) and mixing at a high speed, for about 10 mins until the dough was formed and then at low speed for 5 mins until the dough was properly developed i.e., indicated by dough temperature (30°C) and by the feel of the dough. The dough was then removed from the mixer, kneaded, and proofed at room temperature for about 10 minutes. After proofing, the dough was cut into 75g pieces and shaped manually into a bagel form. The bagels

were then proofed for an additional 5 minutes prior to being boiled in a kettle filled with boiling water containing honey (4 tablespoons in 10L water) until they floated to the surface. Bagels were then removed from the kettle using a wire sieve and drained of excess water. The bagels were coated with sesame seeds on both sides, placed on wire racks and baked for about 18 minutes (9 minutes on each side) in a convection oven at 400°F (Garland Convection Oven (TE3,4CH Commercial Ranges Ltd., Mississauga, Ontario). All ingredients were used at levels suggested in their commercial literature. The ingredients, and their levels of use in the reformulated product, are shown in Table 1 hereinabove.

Thus tests were conducted with guar gum. The effect of such gum (0.2 and 0.6%) on bagel softness is shown in Figure 8. As can be seen, textural shelf life can be extended to about 20 days at the 0.2% level (flour weight basis) whereas at higher levels (0.6%) bagels were stale after 30 days (shown by compressibility test of 0.01 MPa). However, for sensory analysis of products only bagels formulated with 0.6% guar gum were marginally acceptable after 28 days at ambient temperature compared with the 3 day shelf life of non reformulated bagels.

Tests were also conducted with enzymes. They showed that enzymes have a beneficial effect on crumb firmness, i.e., they delay the staling process. While non-reformulated bagels had a sensory shelf life of only 3 days, it was possible to extend the same sensory shelf life of reformulated bagels to up to 42 days. This can be attributed to the ability of these enzymes to "cut" the amylase and amylopectin branches of starch resulting in smaller branches which prevents starch-protein interaction. They also create low molecular weight sugars and dextrans improving the water retention capacity of the baked good. Furthermore, enzymes do not result in "stickiness" or "gumminess" in the end product.

Tests were further conducted with HFCS and showed a shelf life of these reformulated bagels for up to 42 days (see example ).

However, during these preliminary reformulation tests, mold growth was visible in all air packaged bagels after 5 to 6 days at ambient storage temperature. This resulted in non edible bagels after only 5 to 6 days.

COMPARATIVE EXAMPLE 2 (Modified Atmosphere Packaging)

As aforesaid, the spoilage problem can be overcome by packaging the reformulated bagels in either 100% CO<sub>2</sub> or with an Ageless type FX100 oxygen absorbent. Mold growth can then be inhibited throughout the 42 day storage period. These results confirm the antimycotic effect of high CO<sub>2</sub> levels and low O<sub>2</sub> levels on mold growth.

Comparison tests were carried out as follows. After baking, bagels were cooled to room temperature and packaged (2 per bag) in Cryovac barrier bags (size 210x210 mm, Cryovac, Mississauga, Ontario, Canada). An Ageless type FX100 oxygen absorbent (Mitsubishi Gas Chemical Co., Tokyo, Japan) was added to each bag to prevent mold growth during storage. All packaged bagels were stored at 25°C for 6 weeks, and monitored for textural and sensorial qualities at regular intervals (days 0, 3, 7, 14, 28 and 42). A flow process of bagel preparation is shown in Figure 1.

For the purpose of comparison, the antimycotic effect of various gas atmosphere on mold growth on bagels was determined and the results are shown in table 11.

Table 11 Effect of packaging conditions on mold spoilage of bagels

Dough flushed with CO <sub>2</sub>	Packaging conditions	Days to visible mold growth
A	+	100% CO <sub>2</sub>
B	+	Ageless FX absorbent
C	-	100% CO <sub>2</sub>
D	-	Ageless FX absorbent
E	-	Air

The results for textural and sensory changes throughout storage are summarized in Table 12. Shelf life in days was determined from graphical results when a compressibility of 0.01 MPa and a sensory score of <3 was reached.

Table 12: Effect of packaging conditions on textural and sensory shelf life of bagels.

	Dough flushed with CO <sub>2</sub>	Packaging atmosphere	Texture	Shelf life
				Sensory --
A	+	100% CO <sub>2</sub>	~14	~14
B	+	Ageless FX	~14	~14
C	-	100% CO <sub>2</sub>	~42	~21
D	-	Ageless FX	~28	~28
E	-	Air	<7	<7

Air packaged bagels (test E) were stale in less than 7 days as observed previously. Flushing bagels with 100% CO<sub>2</sub> during mixing and subsequently packaging in 100% CO<sub>2</sub> (test A) or with oxygen absorbents (test B) had little effect on either the textural or sensory shelf life. Indeed, bagels were staler than non flushed bagels packaged in either CO<sub>2</sub> or with an oxygen absorbent (tests C and D).

Bagels packaged under 100% CO<sub>2</sub> (test A) had a compressibility of 0.009 after 42 days at room temperature i.e., within the "staling standard" of 0.01 MPa. However, while textural shelf life was acceptable, bagels were rejected after 21 days again due to sharp acidic taste probably caused in dissolution of headspace CO<sub>2</sub> in the aqueous phase of the product.

Finally, bagels packaged with an oxygen absorbent (test D) had a textural and sensory shelf life of 28 days.

The above results confirm earlier observation that flushing CO<sub>2</sub> into the dough during the mixing stage does not have a beneficial effect on crumb texture i.e., staling. They also show that packaging bagels in 100% CO<sub>2</sub> is a useful alternative to reformulation to delay staling. While the exact antistaling mechanism of CO<sub>2</sub> is not known,

it may affect the hydrogen capacity of proteins which would have a plasticizing effect on starch-protein interactions. However, such a packaging in a 100% CO<sub>2</sub> also has the drawback of giving a sharp acidic taste to the bagels.

EXAMPLE 1 (Reformulation/MAP)

Bagels were reformulated with the ingredients mentioned hereinabove and packaged in a modified atmosphere packaging so as to monitor their effect on the textural and sensorial qualities of bagels over a 6 weeks period at ambient storage temperature (25°C).

Based on this initial study, the estimated shelf life of bagels for all reformulated products stored at 25°C are shown in Table 13. The textural shelf life was based on the time (days) to reach a compressibility of 0.01 MPa. While sensory shelf life was based on time (days) to reach an overall acceptability score of <3. It is evident from these results that certain ingredients may result in a desired textural shelf life of 42 days, yet have a lower sensory shelf life, and vice versa.

However, certain formulations involving enzymes (Superfresh, and Megafresh at the 0.150.2% level), gums (algin) and HFCS (liquid) resulted in a 42 day extension in textural and sensorial shelf life of bagels.

EXAMPLE 2 (reformulation/MAP)

This example indicates the shelf life span of bagels reformulated with Novamyl enzyme alone and packaged in MAP.

Novamyl is a genetically modified maltogenic amylase produced by a genetically modified strain of *Bacillus subtilis* (host) which has received the gene for maltogenic amylase from a strain of *Bacillus*

stearothermophilus. When used at a level of 0.031% (flour weight basis) it had a pronounced effect on the textural shelf life of bagels (Figure 5). At the end of the 6 week storage period, bagel texture had changed very little (from 0.006 MPa to 0.007 MPa) over this time period. This was

Table 15: Summary of shelf lives from different formulations..

Formulation	Level of use <sup>1</sup>	Sensory <sup>2</sup> Days	Textural <sup>3</sup> Days
<b>Enzymes:</b>			
Novamyl	0.031	28	42***
	0.047	42	14***
<b>Superfresh</b>			
	0.1	28	42***
	0.15	42	42***
	0.2	42	42***
<b>Megafresh</b>			
	0.1	28*	42***
	0.15	42*	42***
	0.2	14*	28***
<b>Gums:</b>			
Guar <sup>a</sup>	0.2	3*	20**
	0.6	28*	30**
<b>Xanthan</b>			
	0.2	14**	12*
	0.6	3**	25*
	1	3**	12*
<b>Locust bean<sup>b</sup></b>			
	0.2	3	7***
	0.6	7	42***
	1	3	25***
<b>Agar<sup>b</sup></b>			
	0.2	14***	40
	0.6	7***	20
	1	7***	35
Cellulose 40 <sup>c</sup>	1	3	14
Cellulose 300	1	7	25
Cellulose 900	1	7	25
Methylcellulose	1	28	30
<b>Algin</b>			
	0.2	42	42**
	0.6	42	28**
	1	28	28**
<b>Pectin</b>			
	0.2	14	10***
	0.6	28	40***
	1	28	32***
<b>Syrups:</b>			
HFCS Liquid	50	42	42***
	100	28	35***
<b>HFCS Granular<sup>c</sup></b>			
	50	7	35***
	100	14	42***
<b>Flours:</b>			
Rice <sup>c</sup>	25	3	42
Barley <sup>b</sup>	25	3	42**
Corn	50	3	42
<b>Surfactants:</b>			
Atlas p51	0.25	-	14***
	0.375	-	14***
<b>Atlas SSL</b>			
	0.25	-	14***
	0.375	-	7***
<b>Control</b>		3	3

1: % flour basis except for high fructose syrup; % sugar replacement.

2: When a product score was of 3 or above.

3: When product score was of 0.01 or below.

\* \*\* \*\*\* significant with p<0.05, 0.005, 0.0005.

a, b, c when compressibility and sensory have a correlation >75%, >50%, >25%.

well below the textural standard of 0.01 MPa used as an indicator for staling. However, higher levels (0.047%) did not result in an improved textural shelf life. Indeed, product was regarded as stale after about 14 days as indicated by a compressibility test of 0.01 MPa (Figure 5).

The results for the sensory scores of bagels reformulated with Novamyl are shown in Table 14. Based on a "cut-off" acceptability score of 3, it is evident that bagels reformulated with 0.031% Novamyl, had a sensory shelf life of 28 days which is interesting. Thus, while objective measurements resulted in a shelf life of >42 days, product had a stale flavour and odour after 28 days and was considered "stale".

Table 14: Sensory results for Novamyl enzyme.

Novamyl	Sensory Analysis												Overall	
	Odor				Flavor				Texture					
	Days of Storage				7 14 28 42				7 14 28 42					
0.031%	7	14	28	42	7	14	28	42	7	14	28	42	3.8 3.7 3.7 2.8	
	3.6	3.7	3.8	3.5	3.8	3.8	3.8	2.2	3.8	3.7	3.8	2.7		
	+/-	0.9	1.2	0.7	0.8	1	0	0.7	0.9	1	0.5	0.7	1.5	0.9 0.5 0.8 1.1
0.047%	3.6	3.4	3.7	3.4	3.2	3.2	3.2	3.5	3.1	2.9	2.5	2.5	3.4 3.6 3.7 3.3	
	+/-	0.8	0.8	1	1	1	0.7	1.1	0.8	1	1	0.8	1.2	
													1 0.5 1 0.8	

Average of 5 replicates followed (below) by its Standard Deviation.

The compressibility results were highly significant with a p-value of <0.0005 (normally a p-value of <0.05 is considered significant). However, the sensory results were not significant and this is mainly due to the nature of the sensory analysis and the difficulty of the judging task. The p-value measures the relation between the variables and the outcome. When the p-value is 0.05, the results are considered statistically significant, i.e., indicating that the results are not due to chance, but there is a real relation between the days of storage, the level used and compressibility outcome. Furthermore, as expected less than 25% correlation was observed between compressibility and the sensory results, showing once more that even

if texture is an important cause of sample rejection, flavour and odour still influence panellist's perception of freshness.

EXAMPLE 3 (reformulation/MAP)

Similar trends were observed for bagels reformulated with Superfresh enzymes and packaged in MAP.

Superfresh is a mixture of fungal and bacterial amylases which act by hydrolyzing the (1,4) glycosidic linkages of starch by hydrolyzing maltose units into simple sugars. Its effect on the textural and sensorial shelf life of bagels at levels ranging from 0.1 to 0.2% (flour weight basis) are shown in Figure 6 and Table 15. At lower levels of use (0.1%) firmness was fairly constant over the storage period. At higher usage levels (0.15-0.2%), firmness measurements increased slightly from an initial level of 0.006 MPa but were well below the "staling standard" of 0.01 MPa after 42 days. Sensory results showed that optimum results could be achieved with 0.15 or 0.2% Superfresh in the formulation (Table 15), i.e. a textural and sensorial shelf life of 6 weeks was possible using this level of enzyme in the reformulated product.

Table 15 : Sensory results for Superfresh enzyme.

Superfresh	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage															
0.1%	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
	3.9 +/-	3.7 0.6	3.6 0.4	3.4 1.1	3.8 0.5	3.7 0.4	3 1.5	2.6 0.8	3.4 1	3 0.8	3.2 1.3	2.6 1.1	3.9 0.6	3.5 0.5	3.2 1.3	2.8 0.8
0.15%	3.2 +/-	3 0.4	3.2 0.7	3.6 0.8	4 0.3	2.6 0.8	2.8 1.1	2.8 1.5	4 0.6	3.2 0.8	3.2 1	3.8 1.3	3.9 0.5	3.2 0.4	3.6 1.1	3.4 1.3
	3.6 +/-	3.6 0.9	3.8 1.1	3.4 0.5	3.7 1	3.6 1.1	3.6 1.1	3.2 1.3	3.4 1.1	3.2 0.8	3 1	3.2 1	3.6 1	3.4 1.1	3.2 0.8	3.4 0.5

Average of 5 replicates followed (below) by its Standard Deviation.

Superfresh followed the same trend as Novamyl i.e., the compressibility results were highly significant with a p-value <0.0005, while the sensory results were not significant. The correlation between the compressibility and the sensory was also less than 25%.

EXAMPLE 4 (reformulation/MAP)

Tests were carried out on bagels reformulated with Megafresh enzymes and packaged in MAP.

The effect of Megafresh, a bacterial  $\alpha$ -amylase and glucotransferase enzyme system, on staling is shown in Figure 7 and Table 16. At the lower level of use (0.1%) bagels had a compressibility measurements of 0.006 MPa after 42 days. At the 0.15% level, results were similar to those obtained with 0.15% Superfresh and 0.031% Novamyl i.e., products became slightly firmer throughout the 42 days storage period. At the higher level of use (0.2%) bagels reformulated with Megafresh reach their maximum firmness after 35 days (Figure 7). Sensory analysis showed that optimum results were obtained using 0.15% Megafresh i.e., a textural and sensorial shelf life of 6 weeks was possible using this level of enzyme in the reformulated product (Table 16).

Table 16: Sensory results for Megafresh enzyme.

Megafresh	Sensory Analysis															
	Odor				Flavor				Texture				Overall			
	Days of Storage				7 14 28 42				7 14 28 42				7 14 28 42			
0.1%	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
	3.6 +/- 1	3.6 0.5	3.6 0.8	.. —	3.3 1.4	3 1	3 0.7	— —	3.5 1.1	3.4 0.5	3.2 1	— —	3.4 1.3	3 0.7	3 0.7	— —
0.15%	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
	3.7 +/- 0.6	3.8 0.6	3.8 0.8	3.6 0.5	3.9 0.8	3.4 1.1	3.4 0.5	3 0.7	4 1.1	3.4 0.5	3.4 0.5	— 0.8	3.8 0.7	3.4 0.5	3.4 0.5	3.4 0.5
0.2%	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
	3.4 +/- 0.7	3.4 0.5	3.4 1.1	— —	2.8 0.7	2.8 0.8	2.8 1.4	— —	2.8 0.9	2.8 0.8	2.8 1.3	— —	3 0.8	3 0.7	2.8 0.8	— —

1. The sensory was interrupted due to mold growth.

Average of 5 replicates followed (below) by its Standard Deviation.

Compressibility results were again highly significant with a p-value however the sensory results were also significant with a p-value  $<0.005$ . However, here again, statistically, no correlation was found between the compressibility and sensory results.

EXAMPLE 5 (reformulation/MAP)

This example studies the effect of different gums in combination with MAP on the textural and sensory shelf life of bagels.

Texturally, products were rejected after day 12 at lower and upper levels of xanthan gum i.e., 0.2 and 1%. However, at the 0.6% level, bagels had a textural shelf life of about 25 days. Sensorially, however, products were rejected after 7 to 14 days for all levels of xanthan gum used. Favourable compressibility results were observed for bagels reformulated with locust bean gum with the best results being obtained at the 0.6% level of use. However, with the exception of odour scores, products containing locust bean gums, were rejected by panellists after 7 days of storage.

The effect of two levels of guar gum (0.2% and 0.6%) on bagel softness is shown in table 17 and figure 8. Textural shelf life could be extended to about 20 days at the 0.2% level (flour weight basis) whereas at higher levels (0.6%) bagels were stale after about 30 days (shown by compressibility test of 0.01MPa). However, for sensory analysis of products only bagels formulated with 0.6% guar gum were marginally acceptable after 28 days at ambient temperature.

Table 17: Sensory results for guar gum.

Guar	Sensory Analysis												Overall**							
	Odor				Flavor				Texture*											
	Days of Storage																			
0.2%	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42				
	3.2	3.2	3	---	2.7	2.7	2.3	---	2	2	2.8	---	2.5	2.2	2.2	---				
0.6%	0.9	0.5	0.7	---	0.9	0.9	1.4	---	0.8	0.8	1.4	---	1	0.5	0.4	---				
	3.2	3.2	3	---	3.2	3.2	3	---	3.2	3.5	3	---	3.2	3.2	3	---				
+/-	0.5	0.5	0.8	---	0.5	0.5	0.8	---	0.9	0.5	0	---	0.5	0.9	0	---				

1. The sensory was interrupted due to mold growth.

\*\* significant with  $p<0.05$ , 0.005, 0.0005.

Average of 5 replicates followed (below) by its Standard Deviation.

#### EXAMPLE 6 (reformulation/MAP)

The effect of HFCS (liquid or granular) as sugar replacement in the bagel formulation is shown in Figure 9 and Table 18. Both granular and liquid HFCS had a significant effect on crumb staling as shown by compressibility tests (Figure 9).

After 42 days, products reformulated with liquid HFCS (50%) were almost as fresh as day 1 bagels while bagels containing granular HFCS (100%) were only slightly firmer than day 1 bagels. However, while higher levels also delayed firming, products were very sweet and sticky due to the hygroscopic nature of HFCS. From a sensory viewpoint, only the 50% liquid HFCS gave acceptable scores with sensory shelf life being acceptable at the end of the 42 days storage period. Thus, HFCS at this level has the potential to delay staling and to produce an organoleptically acceptable product. High fructose corn syrups compressibility results were highly significant with p-values of 0.0005, while the sensory results were not significant, i.e., similar results to enzymes and gums.

Table 18: Sensory results for high fructose corn syrup.

High Fructose Corn Syrup	Sensory Analysis															
	Odor				Flavor*				Texture				Overall			
	Days of Storage															
	7	14	28	42	7	14	28	42	7	14	28	42	7	14	28	42
Liquid 50% <sup>1</sup> +/-	3.5 0.4	3.6 0.5	3.5 0.7	3 0.7	2.0 0.5	3.0 0.5	3.4 0.5	3 0	3 0.0	3.4 2	3 0.7	3 0.7	2.5 2	3.2 0.8	3 0.7	3 1
Liquid 100% +/-	3.5 1	3.5 1.2	3.2 1	3 0.5	3.3 0.9	3.5 1.5	3 1.5	2.5 1.4	3.0 0.5	3.2 1.5	3 1.2	2.5 0.7	3.5 0.7	3.2 1.5	3 1.1	2.0 0.7
Granular 50% +/-	3.7 1	3 1.2	2.8 1	2.6 0.5	3.7 0.9	2.8 1.5	2.4 1.5	2 1.4	2.7 0.5	2.0 1.5	2 1.2	2 0.7	3 0.7	2.0 1.5	2.4 1.4	2 1.7
Granular 100% +/-	3 1.1	3.3 0.5	3 0.7	2.6 0.5	3.2 0.9	2.8 0.8	2.4 0.8	2 0.7	2.0 1.2	2.2 0.8	2 0.7	2 0.7	3.2 1.2	3 0.8	2.8 0.5	2 1

1. Based on a sugar replacement basis.

The products with high fructose corn syrup were sweeter and stickier than the ones using sugar only.

\* \*\* \*\*\* significant with p<0.05, 0.005, 0.0005.

Average of 6 replicates followed (below) by its Standard Deviation.

## CLAIMS:

1. A method for delaying staling in a bakery product containing a dough made of a flour, for up to 42 days, characterized in that the method comprises the steps of:

-reformulating the dough by adding to the flour at least one ingredient for delaying firmness of the bakery product;

-baking the reformulated dough to form the requested bakery product;  
and

-packaging the bakery product in a modified atmosphere to prevent microbial spoilage.

2. A method as in claim 1, characterized in that said at least one ingredient added to dough during the reformulation step is selected from the group consisting of enzymes, gums and high fructose corn syrups.

3. A method as in claim 2, characterized in that said at least one ingredient is an enzyme selected from the group consisting of genetically modified maltogenic  $\alpha$ -amylases, fungal and bacterial  $\alpha$ -amylases and glucotransferases.

4. A method as in claim 3, characterized in that said at least one ingredient consists of a genetically modified maltogenic  $\alpha$ -amylase used in an amount ranging from 0.10 to 0.20% by weight with respect to the total flour weight.

5. A method as in claim 3, characterized in that said at least one ingredient consists of a mixture of fungal and bacterial  $\alpha$ -amylases used in an amount ranging from 0.031 to 0.047% by weight with respect to the total flour weight.

6. A method as in claim 3, characterized in that said at least one ingredient consists of a mixture of a bacterial  $\alpha$ -amylase and a glucotransferase used in an amount ranging from 0.10 to 0.20% by weight with respect to the total flour weight.
7. A method as in claim 2, characterized in that said at least one ingredient consists of a gum selected from the group consisting of Guar, Xanthan, Locust Bean and Agar.
8. A method as in claim 7, characterized in that the gum is guar and is used in an amount ranging from 0.2 to 0.6 by weight with respect to the total flour weight.
9. A method as in claim 2, characterized in that said at least one ingredient consists of a high fructose corn syrup in a liquid or solid state.
10. A method as in claim 9, for use in the preparation of a bakery product containing sugar, characterized in that the high fructose corn syrup is in a liquid state and is used instead of said sugar.
11. A method as in claim 10, characterized in that the liquid high fructose corn syrup is used in an amount of 50% with respect to the total sugar weight.
12. A method of claim 9, for use in the preparation of a bakery product containing sugar, characterized in that the high fructose corn syrup is in a solid state and is used instead of said sugar.
13. A method of claim 12, characterized in that the solid high fructose corn syrup is used in an amount of 50% with respect to the total sugar weight.

14 A method as in claim 2, characterized in that the packaging step is carried out by packaging the baked product in a mixture of gases.

15 a method of claim 14, characterized in that the mixture of gases consist of N<sub>2</sub> and CO<sub>2</sub>.

16. A method as in claim 2, characterized in that the baking product is packaged under 100% CO<sub>2</sub>.

17. A method as in claim 2, characterized in that the baked product is packaged in the presence of an oxygen absorbent.

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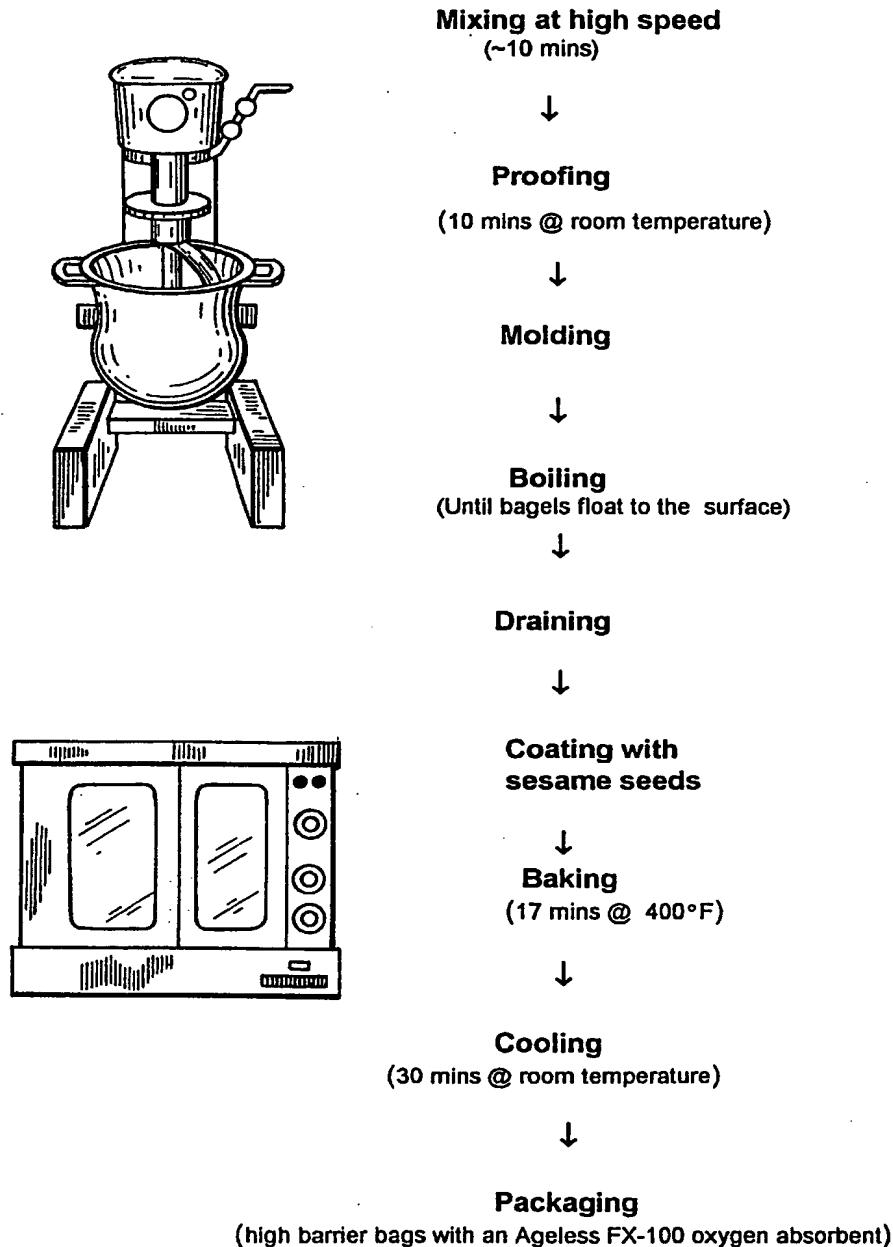


Figure 2.1: Bagel preparation.

FIG. 1

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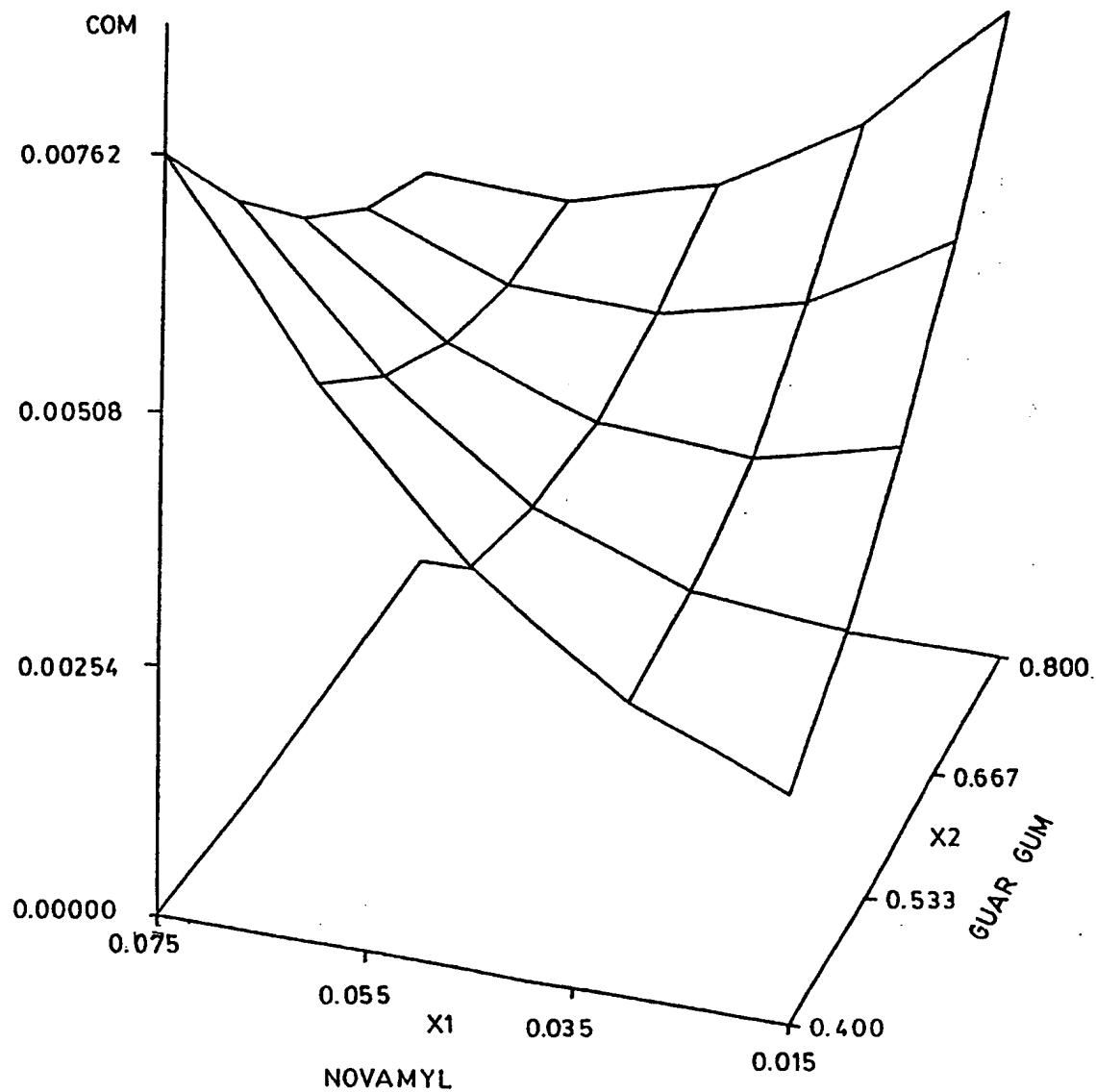


FIG. 2

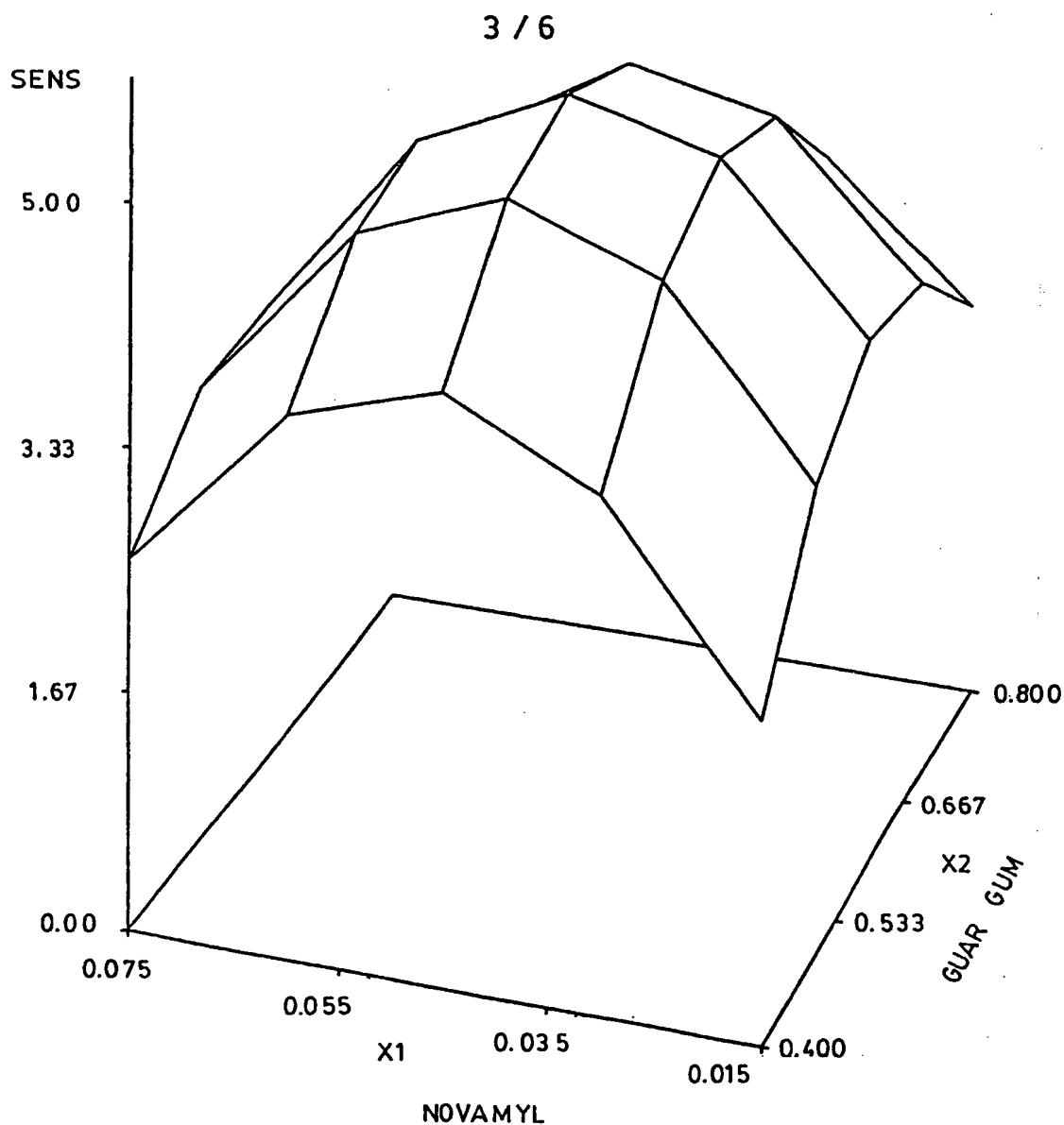
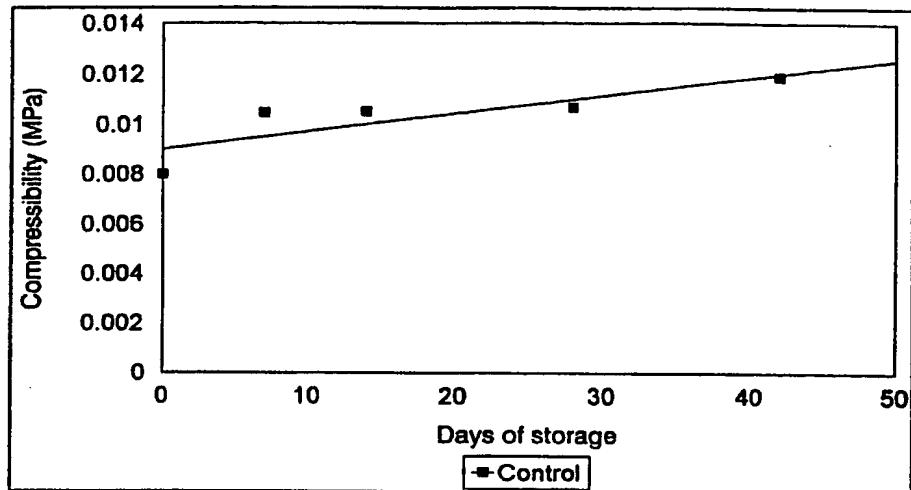
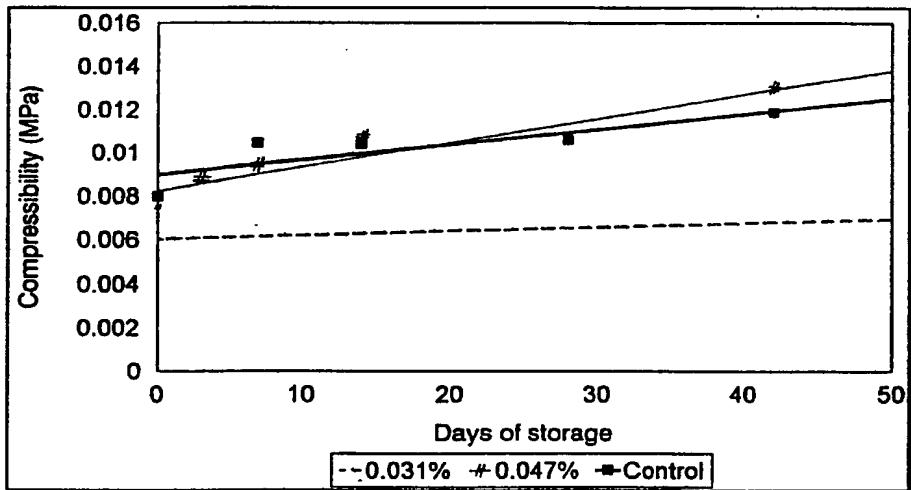


FIG. 3

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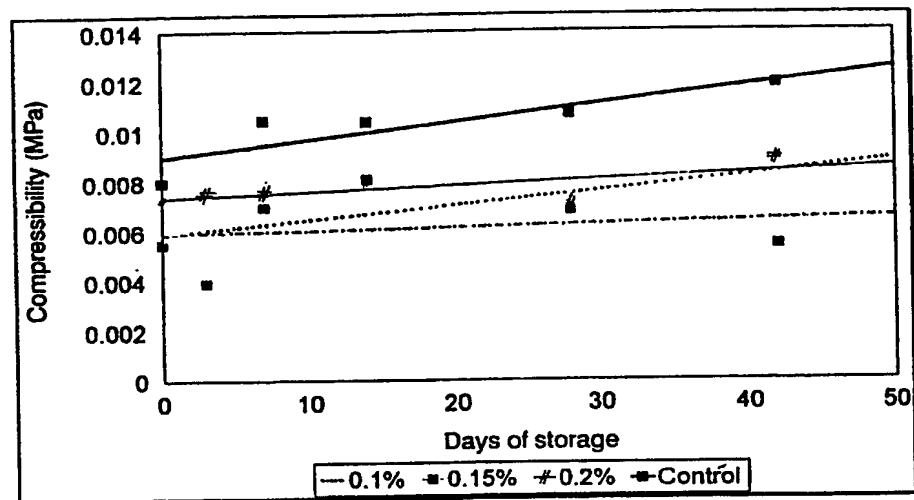
Compressibility results for control bagels.

FIG. 4

Compressibility results for Novamyl enzyme.

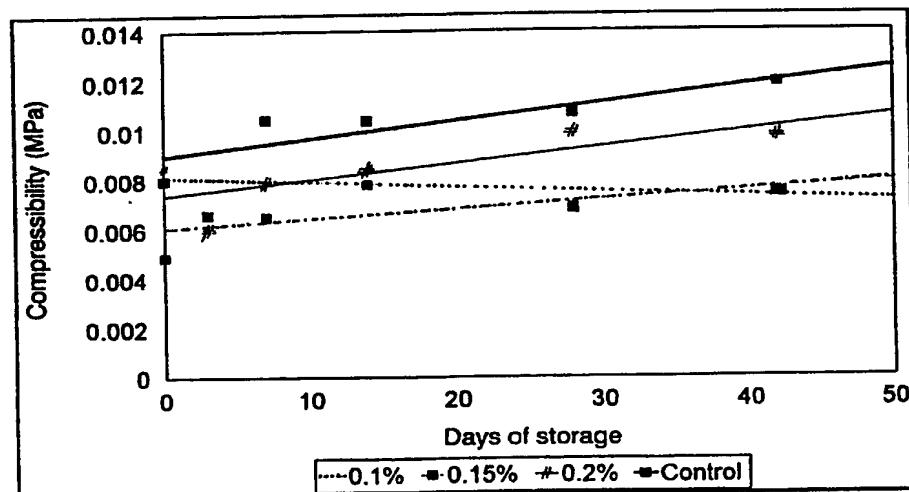
FIG. 5

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Compressibility results for Superfresh enzyme.

FIG. 6



Compressibility results for Megafresh enzyme.

FIG. 7

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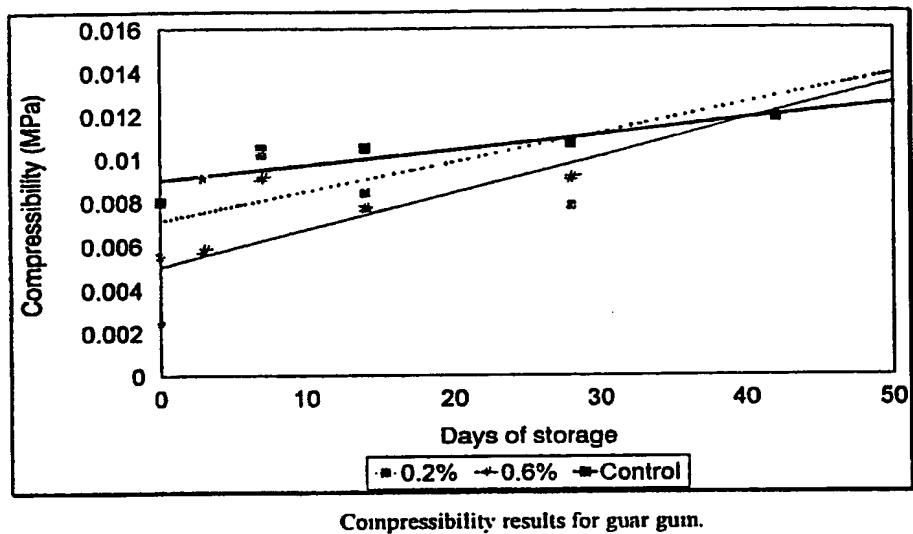


FIG. 8

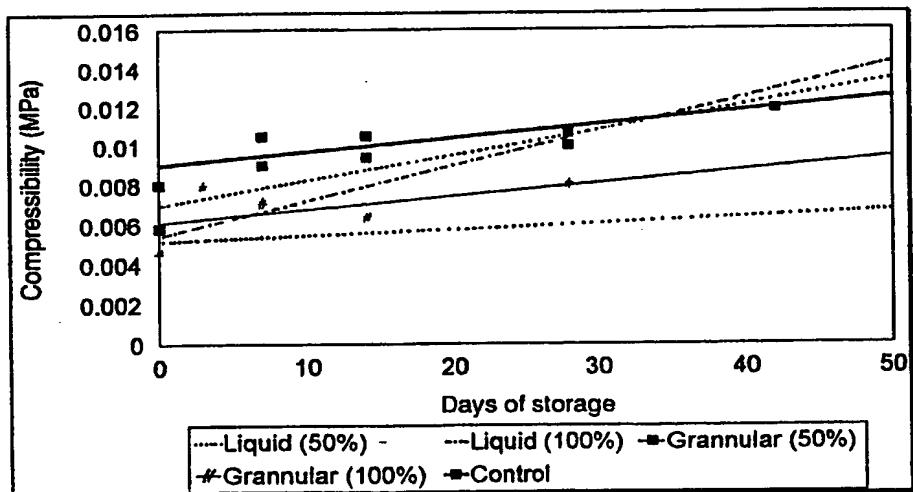


FIG. 9

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA 97/00800

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A21D15/00 A21D8/04 A21D2/18

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A21D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 654 218 A (JOSE MIGUEL POVEDA S A JOMIPSA) 24 May 1995 see claims ---	1,7, 14-16
Y	WO 91 04669 A (NOVONORDISK AS) 18 April 1991 see claims ---	1-4, 14-16
Y	GB 2 236 240 A (E B I FOODS LIMITED) 3 April 1991 see page 4, line 25 – page 6, line 31 ---	1-4, 14-16
Y	US 5 472 724 A (WILLIAMS KEVIN P ET AL) 5 December 1995 see the whole document ---	1,2, 14-16
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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1

Date of the actual completion of the international search

Date of mailing of the international search report

10 February 1998

18/02/1998

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**INTERNATIONAL SEARCH REPORT**

International Application No  
PCT/CA 97/00800

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